

Dimorphic Nature in Stigma-Anther Separation of *Prunus nipponica* (Rosaceae)

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Stigma height, anther height and stigma-anther separation were studied to examine their dimorphic nature in two populations of *Prunus nipponica* occurring in sub-alpine zone of Mts. Norikura and Utsukushigahara, central Honshu of Japan. The stigma and anther heights considerably varied among the plants and each of the characters showed normal frequency distributions within populations. However, stigma-anther separation showed a bimodal distribution. Based on this floral character the populations concerned were tentatively divided into two discrete floral morphs: long (L)-styled morph and homo (H)-styled morph. Each of the two morphs was highly constant within a plant and did not change its floral nature for over two years. Artificial pollination experiments indicated that the two morphs were strongly self-incompatible and crosses between the same morphs (H x H or L x L) produced seeds in higher percentage. These results suggest that the dimorphic nature in *P. nipponica* does not exhibit an ordinary heterostyly but may be regarded as an example of herkogamous polymorphism. We briefly discuss on the possible adaptive significance of dimorphic natures of the flowers exhibited by *P. nipponica*.

Key words: Dimorphic, herkogamous polymorphism, heterostyly, *Prunus nipponica*, seed sets, self-incompatible, stigma-anther separation

Prunus L. (Rosaceae) is a large genus including approximately 200 species and it mainly occurs in temperate regions of the Northern Hemisphere. In Japan approximately 15 wild species are known within the genus (Ohba 1989). During our field survey, we found that *Prunus nipponica* Matsum. occurring in Mt. Norikura had two readily distinguishable plants in terms of style position relative to anthers in the flowers: plants with a style extruding above the anther level and plants with a style as tall as the anther. However, heterostyly or herkogamous polymorphism has not been

reported for the species (Ohba 1989) and other species of the family so far (Ganders 1979, Barrett 1992, Lloyd & Webb 1992).

Heterostyly is a genetically determined reciprocal herkogamy of stigma and anther height (Darwin 1877, Ganders 1979, Lloyd & Webb 1992). In distylous species the long (L)-styled morph has long styles and short stamens, while the short (S)-styled morph has short styles and long stamens. Pollination studies of the heterostylous species have indicated that reciprocal pollination between the different flower morphs and self-incompatibility within

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a morph are common (Ganders 1979). Heterostyly is known to occur in various genera belonging to 24 families of the flowering plants (Ganders 1979, Barrett 1992). In addition to the ordinary heterostyly, several types of herkogamous polymorphism have been recognized in some species (Lloyd & Webb 1992). For example, stilar dimorphism, in which stigma height differs between two morphs but anther height does not differ, is known in *Chlorogalum angustifolium* (Jernsted 1982) and *Epacris impressa* (O'Brien & Calder 1989). In these species including the ordinary heterostylous species flowers are usually actinomorphic and tubular, and stamens are arranged around the entrance of corolla tube (Ganders 1979, Lloyd & Webb 1992). It is also common to the above species that the flowers are insect-pollinated. *Prunus nipponica* concerned here is presumed to possess the floral traits common to the heterostylous species or the species exhibiting the herkogamous polymorphism, so we aim to make clear the floral and reproductive natures of the species.

In this paper we investigate variations in

stigma and anther heights, stigma-anther separation, and self-incompatibility in order to determine whether the species is heterostylous. We also examine how floral variation is associated with seed sets in a natural population.

Materials and Methods

Plant and study site

Prunus nipponica Matsum. (Rosaceae) is a deciduous small tree growing in high mountain regions (usually sub-alpine zone) in central to northern Honshu and Hokkaido of Japan. It generally blooms from May to July. A flower is actinomorphic, forming a tubular calyx tube, and secretes nectar from the base of a calyx tube. In general, style height has been described as equal to stamen height (Ohba 1989). On preliminary observations we found that two different morphs in stigma height occurred in natural populations (Fig. 1). Therefore variations in stigma height, anther height and stigma-anther separation were examined in two natural populations of Mts. Norikura and Utsukushigahara, central Honshu of Japan.

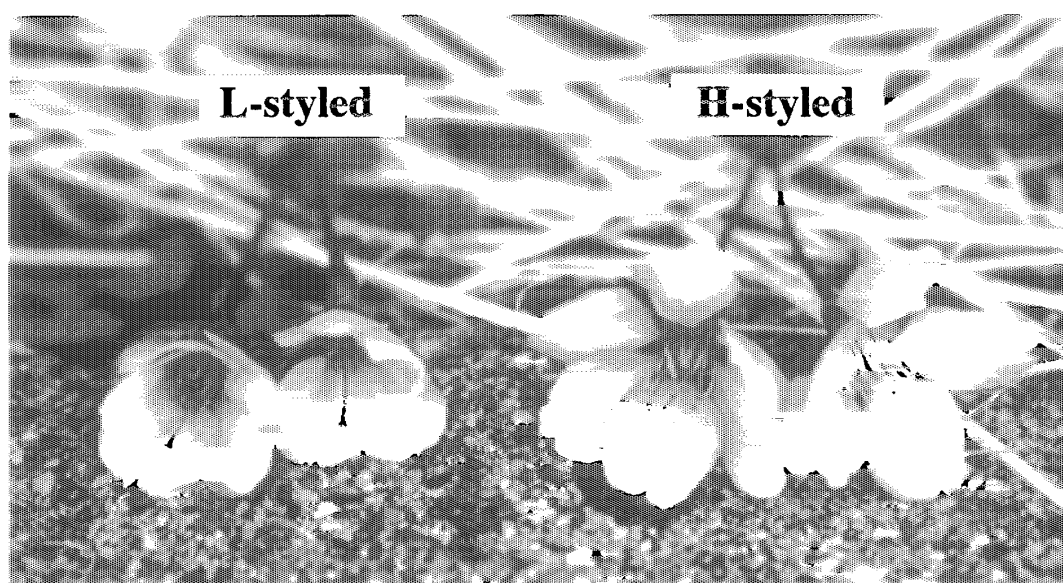


FIG. 1. Long styled (L-styled) and homo styled (H-styled) flowers found in the Norikura population of *Prunus nipponica*.

Our field investigations were carried out along the Sanbondaki trail, leading to the summit of Mt. Norikura (the Norikura population, altitude 1600m-2400m), and at Mt. Utsukushigahara (the Utsukushigahara population, altitude 1800m-1880m). In the former population, evergreen coniferous trees of *Abies veitchii* Lindl. and *A. mariesii* Mast. are common. Broad-leaved deciduous trees, such as *Betula ermanii* Cham., *Sorbus commixta* Hedl., *S. matsumurana* (Makino) Koehne, *Acer tschonoskii* Maxim., and *Viburnum furcatum* Blume ex Maxim., also occurred along roadsides and dry slopes. In the latter population, *Larix kaempferi* (Lamb.) Carriere, *Rhododendron japonicum* (A. Gray) Suringar, *Sasa senanensis*

(Franch. et Sav.) Rehder are common.

Measurements on floral variation

Measurements on floral variation were performed in 76 and 58 plants for the Norikura and the Utsukushigahara populations, respectively. In field five fully open flowers were collected from each plant at random and were fixed in FAA. After fixation the five flowers from each plant were measured on their stigma height (a) and anther height (b), as illustrated in Figure 2, and stigma-anther separation ($c = a - b$) was calculated. Mean value of the five flowers examined was used as a representative of each individual.

In the Norikura population, floral variation

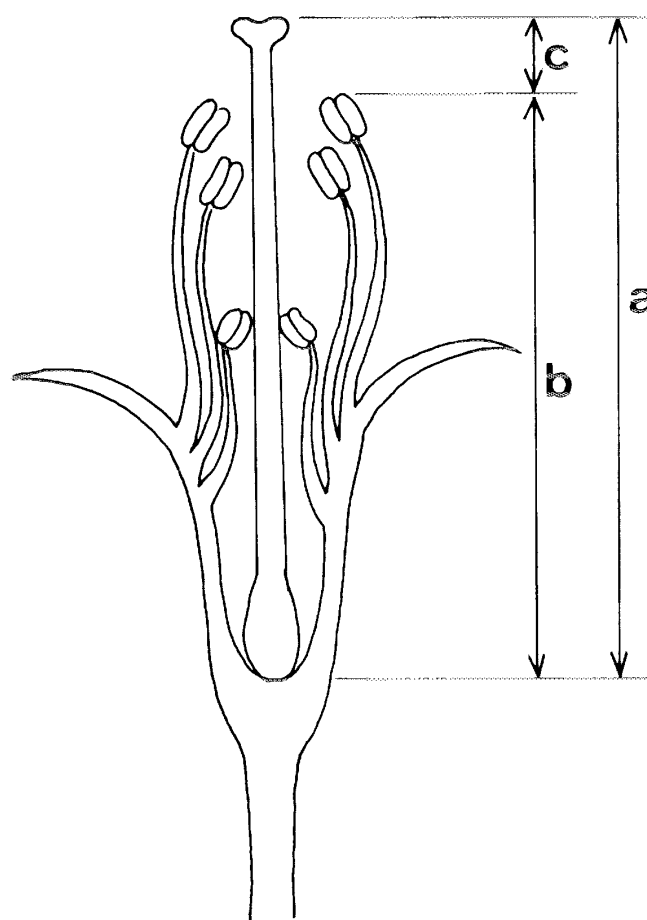


FIG. 2. A part of flower showing morphological measurements. a, stigma height. b, anther height. c, stigma-anther separation.

within a plant was monitored for two years with the nine plants selected at random.

Pollination experiments and seed sets in a natural population

Bagging and artificial self-pollination experiments were carried out to examine the possibility of self-pollination and to confirm the probability of self-compatibility for different flower morphs in the Norikura population. Flower buds positioned on several branches at 1 - 2m were selected randomly in each tree and they were placed in small tea bags (quality of the material: polyethylene and polypropylene, produced by Marusan Co.) to exclude insect pollinators. After anthesis some of the bagged

flowers were left untouched, and the others were artificially self-pollinated and bagged again for a month until fruit maturation. The number of flowers and plants examined are shown in Table 1. Artificial cross-pollination experiments between and within two different flower morphs were also carried out. In this case the distance between the study plants with the different morphs was 20 - 50 m. The flower buds emasculated prior to anthesis were bagged and left untouched for two or three days. After stigma maturation, the emasculated flowers were artificially cross-pollinated and bagged again for a month until fruit maturation (see Table 1). In addition, the amount of seed-set (number of flowers fruited / number of flowers examined;

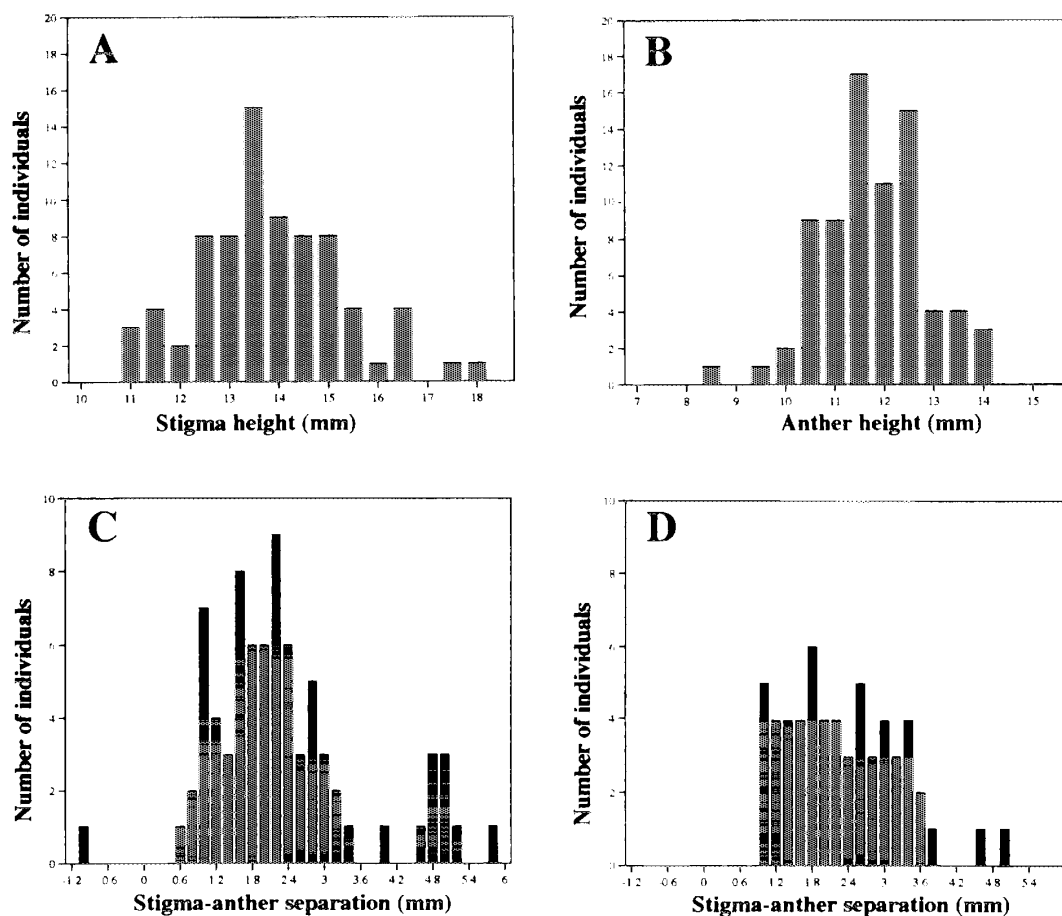


FIG. 3. Frequency distribution of stigma height (A), anther height (B), and stigma-anther separation (C) in the Norikura population ($N = 76$) of *Prunus nipponica*. The frequency distribution of stigma-anther separation in the Utsukushigahara population ($N = 58$) of *Prunus nipponica* is also shown in D.

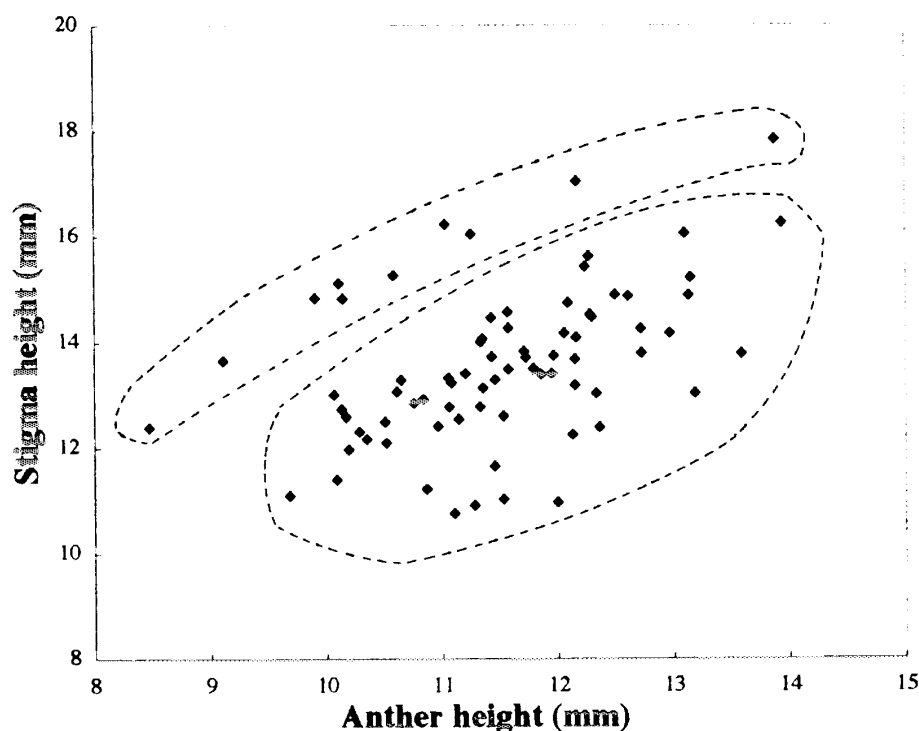


FIG. 4. Relationship between stigma height and anther height in the Norikura population of *Prunus nipponica*.

the flower fruited includes only one seed) by open pollination was examined in a total of 51 plants (trees) at the Norikura population, and average seed set for each plant was calculated. In this case more than 40 flowers per plant were examined.

Results

Variations in stigma height, anther height and stigma-anther separation among plants

Frequency distributions for stigma height, anther height and stigma-anther separation obtained from the Norikura population are shown in Figure 3A, 3B and 3C, respectively. Each of the stigma height and anther height was normally distributed (Normal test for stigma height, $\chi^2 = 2.27$, $p = 0.81$; normal test for anther height, $\chi^2 = 3.44$, $p = 0.63$; ref. Yanai 1998). The stigma-anther separation, however, indicated a bimodal distribution (Normal test, $\chi^2 = 24.33$, $p < 0.001$;

Fig. 3C). A scatter diagram on the relationship between stigma height and anther height in the Norikura population is shown in Figure 4. In this diagram two plant groups were obviously recognized (Discriminant analysis, Mahalanobis' $D^2 = 10.66$, $F = 45.65$, $p < 0.001$; ref. Yanai 1998). Plants that represent longer stigma-anther separation (more than 3.8 mm; $N = 10$, mean 4.82 mm) were grouped into a long (L)-styled morph, while plants that have shorter stigma-anther separation (less than 3.8 mm; $N = 66$, mean 1.67 mm) were grouped into a homo (H)-styled morph (Fig. 3C). In the L-styled morph the stigma is always positioned above the anther levels. In the H-styled morph the stigma is positioned at the same height as the anther levels or slightly above the anther levels.

Similar phenomenon was also observed in the Utsukushigahara population (Fig. 3D). Frequency distribution of the stigma-anther

separation in the Utsukushigahara population was deviated from a normal distribution (Normal test, $X^2 = 7.81$, $p < 0.05$; ref. Yanai 1998), as in that of the Norikura population. In this population the plants with L-styled morphs (more than 4.2 mm; $N = 2$, mean 4.72 mm) were very rare, and the plants with H-styled morphs (less than 4.2 mm; $N = 56$, mean 2.15 mm) are dominant (Fig. 3D).

In supplemental observations no significant differences were found between the H-styled and L-styled morphs in other floral traits, such as stigma size, calyx-tube length, anther size and pollen size.

Floral variation within a plant monitored for two years

Stigma height, anther height, and stigma-anther separation of nine plants in the Norikura population were monitored for two flowering

seasons (Fig. 5). These floral characters were uniform within a plant and did not change style morph for two flowering seasons. In the nine plants examined, we could find no significant differences between the two flowering seasons in each test (Mann Whitney's U-test, $p > 0.002$ for each test; adjusted by Bonferroni adjustment procedure at $p = 0.05$; ref. SISA 1998).

Pollination experiments and seed sets in L- and H-styled morphs

To test the self-compatibility, a total of 160 flowers from nine plants representing H-styled and L-styled morphs were bagged. Furthermore, 51 flowers from two H-styled and two L-styled morphs were artificially self-pollinated. Under these experiments none of the flowers set seeds (Table 1).

Results on artificial cross-pollination experiments in H-styled x H-styled morphs, H-

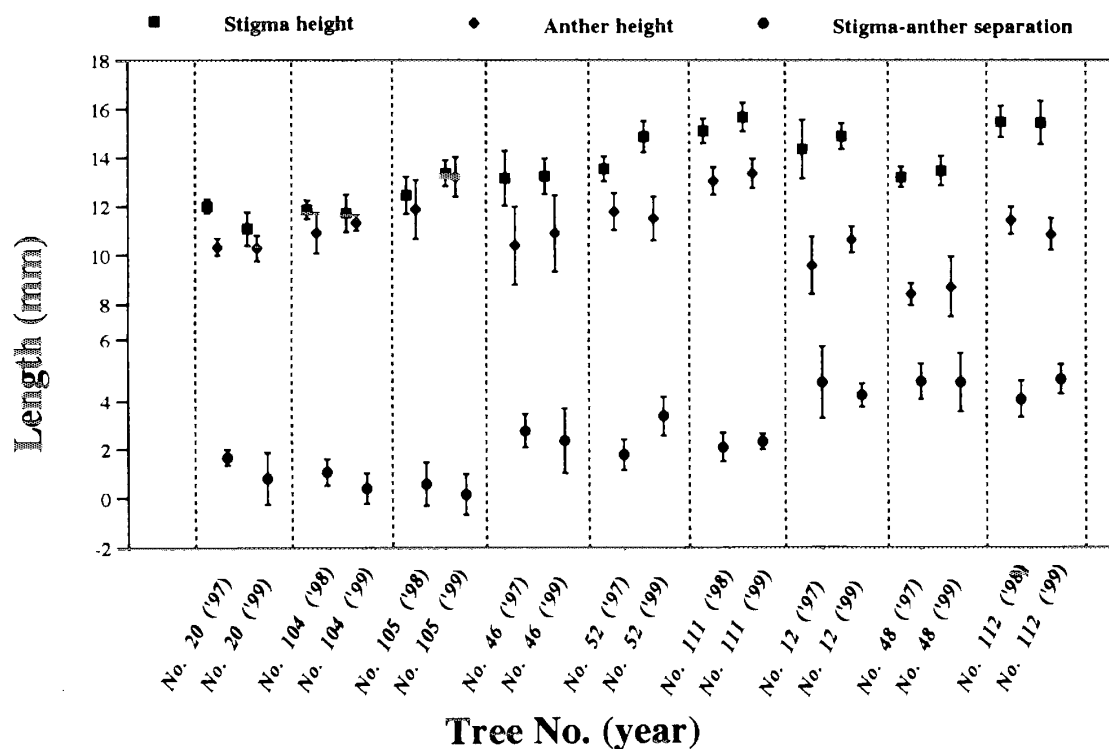


FIG. 5. Mean values of stigma height, anther height and stigma-anther separation in nine plants of the Norikura population monitored for two years. Vertical bars indicated standard deviation. Mann Whitney's U-test was carried out between the two flowering seasons, and all the tests ($N = 27$) were adjusted by Bonferroni adjustment procedure at $p = 0.05$.

TABLE 1. Artificial pollination experiments in H-styled and L-styled morphs.

Treatment	N	No. of flowers examined	Seed set %
<Bagging>			
H	7	132	0
L	2	28	0
<Self-pollination>			
H	2	34	0
L	2	17	0
<Cross pollination> (female x male)			
H x H	16	281	41.6
H x L	9	136	66.2
L x H	6	135	55.6
L x L	4	130	56.9
<Open pollination>			
H	45	3037	17.4 : 13.51 ¹⁾
L	6	403	23.9 : 10.11 ¹⁾

N: number of plants examined. ¹⁾Seed set percentage per plant

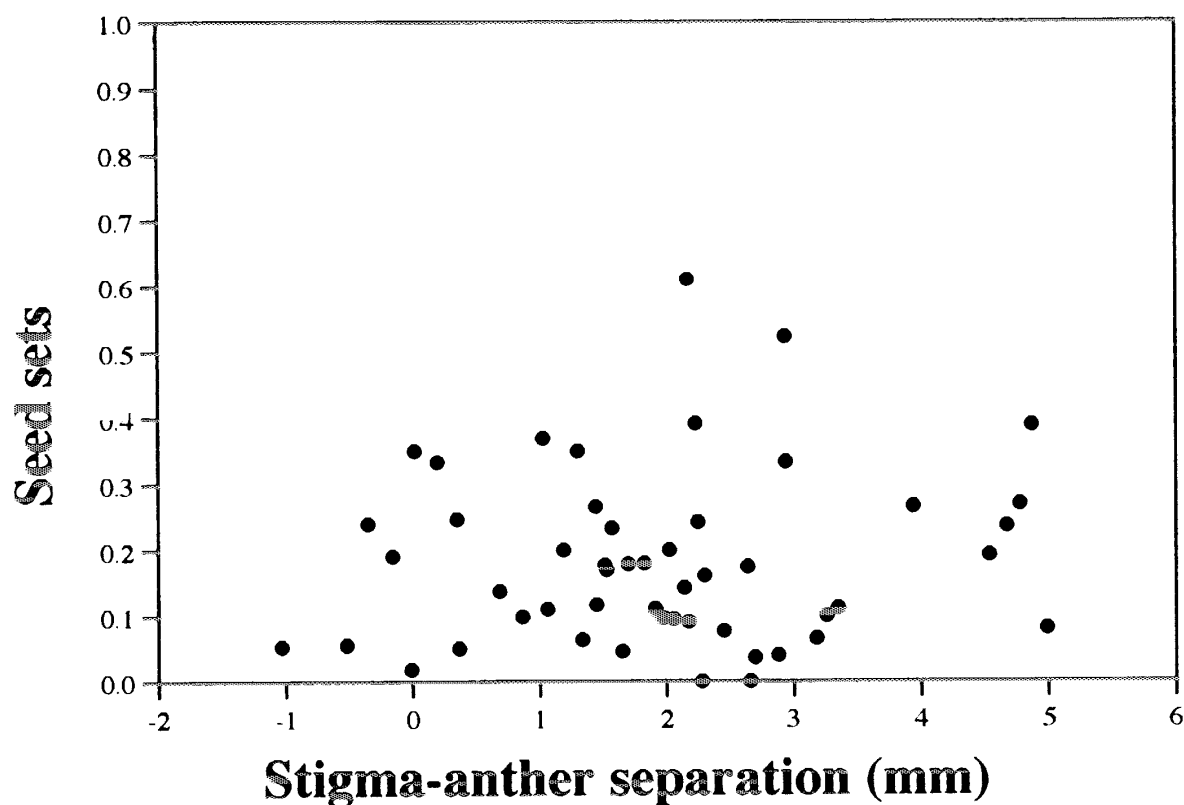


FIG. 6. Relationship between stigma-anther separation and seed set by open pollination examined in 1999. Number of the L- and H-styled plants (trees) examined are 6 and 45, respectively.

styled x L-styled morphs, L-styled x H-styled morphs, and L-styled x L-styled morphs respectively are also shown in Table 1. A significant difference was found among the four treatments in seed set (Chi Square test, $\chi^2 = 24.98$, $p < 0.001$; ref. Ball 1999). The H-styled x H-styled morphs showed the lowest value (41.6%), and the H-styled and L-styled morphs had the highest value (66.2%) in seed set percentages (see Table 1). The L-styled x L-styled morphs also set seeds in higher value (56.9%).

Seed set percentages of H-styled and L-styled plants by open-pollination are shown in Table 1. The seed set percentage of the H-styled plants ($N = 45$) ranged from 0% to 61.1%, while that of the L-styled plants ($N = 6$) ranged from 8.1% to 38.9%. Average seed set percentage of the L-styled plants appears to be higher than that of the H-styled plants, but they did not differ significantly (Mann-Whitney's U-test, $p = 0.105$).

Relationship between stigma-anther separation and seed set under the open pollinated conditions is shown in Figure 6. There was no correlation between stigma-anther separation and seed set (Spearman's correlation coefficient by rank test, $p = 0.376$).

Discussion

The present results indicate that populations of *Prunus nipponica* occurring in Mts. Norikura and Utsukushigahara consist of plants of two discrete floral morphs: long (L) -styled and homo (H) -styled morphs. In the L-styled morph the stigma height is highly above the anther height, and stigmas-anther separation is more than 3.8 and 4.2 mm in the Norikura and Utsukushigahara populations, respectively. In the H-styled morph, on the other hand, the stigma height is the same as the anther height or

slightly above the anther height. In the two populations examined the L-styled morph is obviously less in frequency, and the plants of two different floral morphs are growing side by side without any differences in their growing environmental conditions. In addition, the two floral morphs are highly stable within plants and do not change their style morphs within plants for at least two flowering seasons. Thus the two floral morphs found in *P. nipponica* appear to be determined genetically.

The floral morphs found in *Prunus nipponica* have a resemblance to those of the ordinary heterostyly, especially of the homo-styly caused by the breakdown of heterostyly (e.g., *Amsinckia spectabilis*, Ganders 1979; *Primula* and *Turnera ulmifolia*, Barrett 1992). However, an obvious difference is found between *P. nipponica* and the heterostylous species with the homo-styles. Usually, the morph of homo-style is highly self-compatible (Ganders 1979, Barrett 1992). The H-styled plants found in *P. nipponica* are, however, self-incompatible highly, as being demonstrated by artificial self-pollination. If the species examined here is truly heterostylous, we would expect no seed production in crosses between the same morphs (Ganders 1979, Barrett 1992). In *P. nipponica*, however, artificial crosses between H- and H-styled morphs or between L- and L-styled morphs result in higher seed production (41.6% and 56.9%, respectively; see Table 1). Based on these results we consider that the two morphs in *P. nipponica* do not possess the morphological and incompatible natures associated with the ordinary heterostyly.

The dimorphic nature exhibited by *Prunus nipponica* is similar to the herkogamous polymorphism found in *Epacris impressa* (O'Brien & Calder 1989), *Chlorogalum angustifolium* (Jernstedt 1982) and *Guettarda scabra* (Richards & Koptur 1993). In this type

of herkogamous polymorphism two herkogamous morphs which differ from each other in stigma height but not in anther height are recognized (Lloyd & Webb 1992). However, the two morphs of this type are highly self-compatible, while those of *P. nipponica* are strongly self-incompatible. In general, herkogamous conditions including a reciprocal herkogamy (heterostyly) is regarded to promote out-crossing among the plants (Ganders 1979, Barrett *et al.* 1996). As stated above, *P. nipponica* is self-incompatible, and thus out-crossing is usual for the species. We wonder why *P. nipponica* presents the dimorphic nature in stigma-anther separation within a population. The H-styled morph in *P. nipponica* shows a close proximity of stigma and anthers, and consequently might cause self-pollination mechanically. One possible interpretation is that deposition of self pollen grains on stigma of the same flower might clog the landing of pollen grains from other plants, as suggested in some species (Bawa & Opler 1975, Webb & Lloyd 1986, Kikuzawa 1989). In such circumstances the L-styled morph is presumed to enhance outcrossing without any clogging. The result obtained from the present observation however does not support this interpretation positively since no correlation is found between stigma-anther separation and seed set for the plants of the Norikura population.

Seed sets by open pollination considerably vary within a natural population and their mean values are conspicuously lower, as compared with those of the artificial cross-pollination (see Table 1). Availability of pollinators may be involved in maintaining the dimorphic nature of the species. In preliminary observations, several insects such as bumblebee (*Bombus heaticola beaticola*), small bees (Andrenidae) and hover flies (Syrphidae) were found to visit flowers of *P. nipponica* in the Norikura. Among

them the bumblebee appears to be the most useful pollinator. In order to understand adaptive and evolutionary significance of the dimorphic nature inherent in this species, further investigations on pollen loads and pollination systems of the two morphs will be necessary.

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